Mutation Surveyor® Software combines both automation and accurate variant calling into a single easy-to-use analysis package. The software is compatible with all major capillary electrophoresis systems, allowing for an integrated workflow from importing of raw data to analysis and reporting. The patented anti-correlation technology (US patent 8,086,401) physically compares sample sequence traces to a reference trace, calculating dropping factor, overlapping factor, peak height and signal to noise ratio of each individual peak, providing accuracy up to 99.5% with Phred 20 bi-directional sequences at sensitivity of 5% of the primary peak. The software monitors the mobility of sample traces to the reference to detect INDELs ranging from one bp to 15% of the sample sequence.

Mutation Surveyor analyzes up to 2000 traces of data in less than 15 minutes and provides an unattended pipeline tool that includes a heterozygous INDEL deconvolution tool, performs hyper variable sequence analysis, mutation quantification, mitochondrial analysis, methylation analysis as well as low frequency variant detection.

Mutation Surveyor software, Version 5.0 and beyond, includes a variant knowledge database allowing users to indicate false-positives and other artifacts, regions of significance and high frequency variants by chromosomal position.

Accuracy & Sensitivity

Anti-Correlation Technology: Mutation Surveyor software uses an anti-correlation technology which compares sample sequences to a reference sequence. The patented algorithm detects variants and calculates a mutation confidence score by using peak intensities, dropping factor (vertical), overlapping factor (horizontal base spacing), and signal to noise ratio of each peak. Using this technology with bi-directional data, accuracy is over 99.5% for Phred 20 sequence traces with sensitivity to 5% of the primary peak or 1 part diseased in 19 parts normal when analyzing cancerous samples. Found variants are displayed as peaks for fast and easy review in our mutation electropherograms as shown in Figure 1-center panes.

### Figure 1: Anti-Correlation Technology detects SNPs
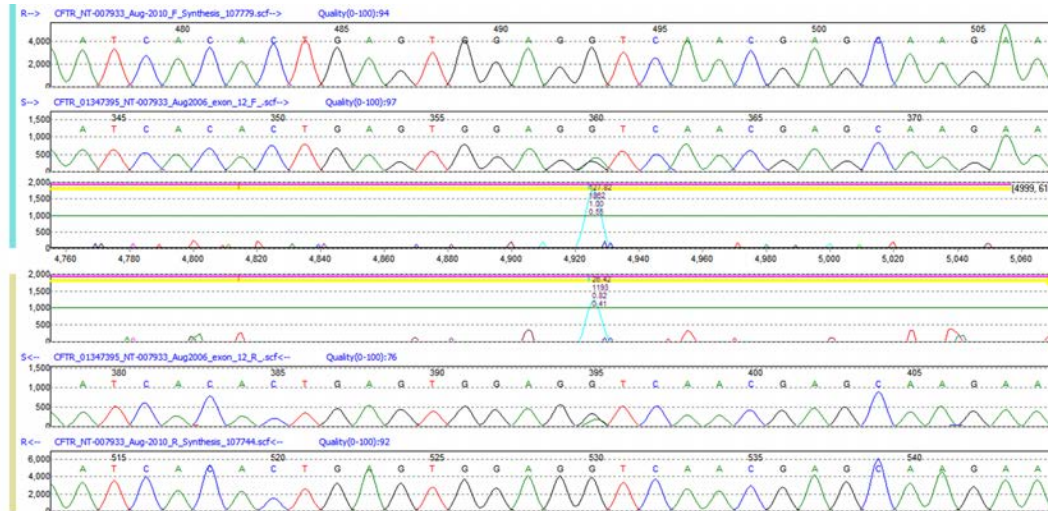
A heterozygous SNP of G>GA is detected in the sample trace. The center mutation electropherograms display the variants as peaks for fast and easy review.

Multi-Step Sequence Alignment INDEL Detection

Mutation Surveyor software utilizes a multistep alignment algorithm to detect INDELs based on mobility shift when the samples are aligned to the reference. The software is capable of aligning sequence traces containing up to 20% variation from the reference. By aligning the traces in 12bp, 6bp, and 3bp-mers to the reference trace, the software detects both small and large INDELs, up to 15% of the sequence trace length.

### Figure 2: Alignment Algorithm Detects Large INDELS
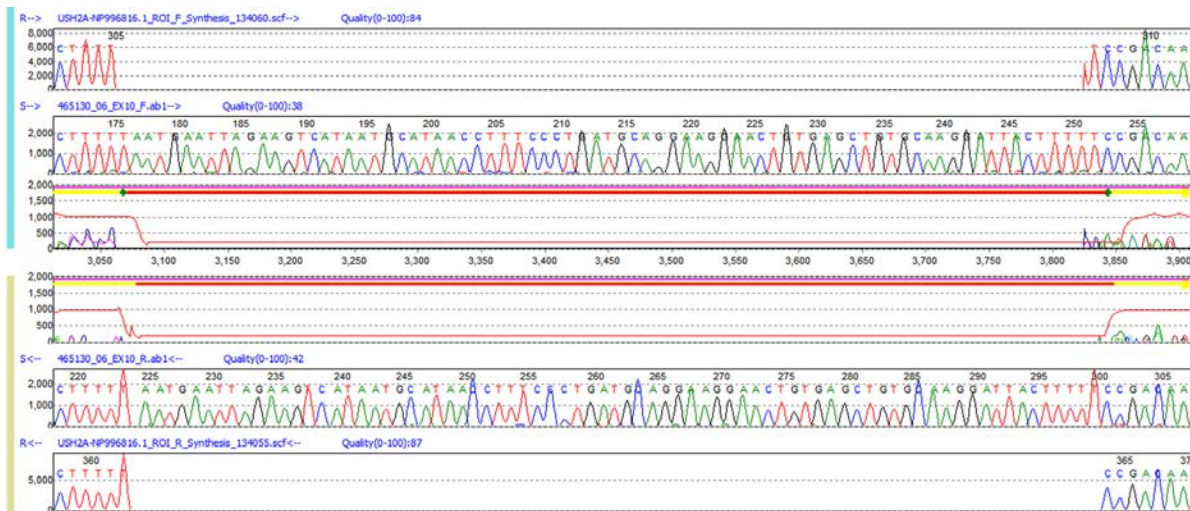
A 76 base pair insertion detected in the sample trace. The reference trace is gapped, and a red bar is placed in the Mutation Electropherogram to indicate the location of the event.

New Variant Knowledge Base

Mutation Surveyor software now includes a variant knowledge base that easily monitors and automates frequent edits and variants found in mutation analysis. The database features include tracking of artifacts or repeatedly deleted mutation calls, such as false positives, tracking of added variants or false negatives and tracking of high frequency variants, with positions stored in chromosomal locations. Also included in the knowledge base is a track manager tool that provides annotated results in the Graphic Analysis Display and Mutation Reports. Annotation from external databases such as dbSNP, COSMIC, causative prediction from dbNSFP as well as custom database information can be added to mutation projects to highlight positions of significance.

Figure 3: Track pane of Graphic Analysis Display showing chromosome coordinates and knowledge base annotation.
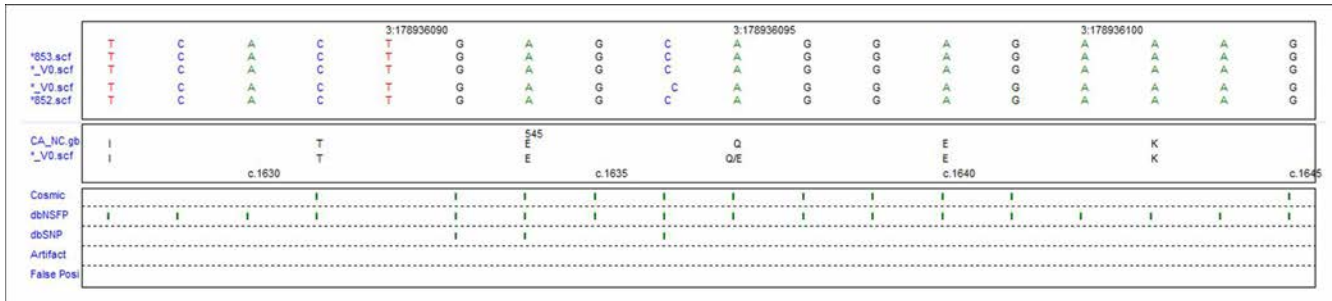
Nucleotide, amino acid, and track panes showing chromosome coordinates and knowledge base annotation. Green tick marks indicate annotation at specific positions.

Figure 4: Mutation Table of Graphic Analysis Display shows chromosome position and knowledge base annotation
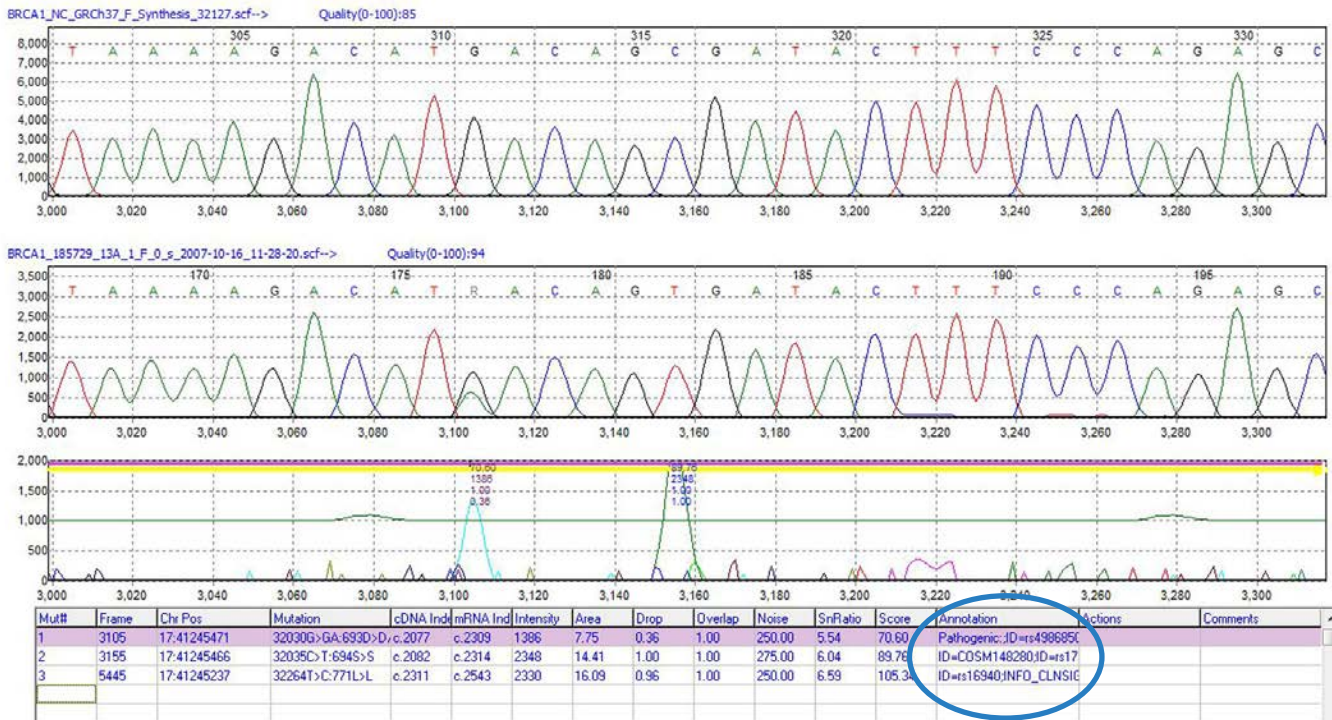

The Mutation Table of the Graphic Analysis Display shows chromosome position, mutation information, and knowledge base annotation.

Heterozygous INDEL Deconvolution

Mutation Surveyor Heterozygous INDEL Deconvolution tool automatically deconvolutes the heterozygous mutant trace from the wild-type trace. Once the INDEL is detected, it is indicated by a horizontal brown bar in the Graphic Analysis Display as shown in Figure 5.

Figure 5: Heterozygous INDEL Sequence Pattern
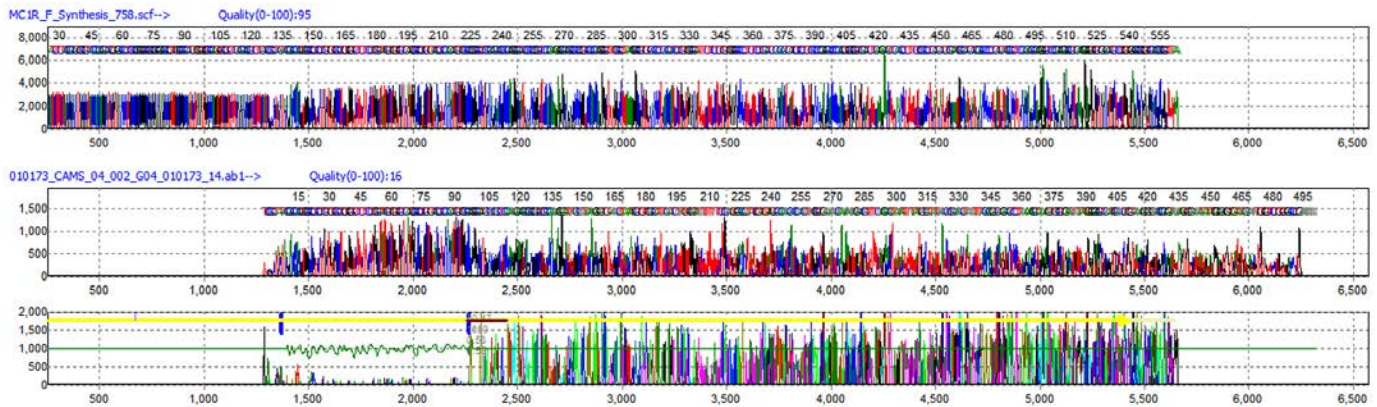
This is a typical pattern for a heterozygous INDEL. After the INDEL position, there is a pattern of double traces, one wild-type and one mutant trace with the INDEL. The Mutation Electropherogram shows that there is less noise within the region of the sample trace that matches well to the reference trace. The noisy region, after the heterozygous indel, is attributed to the overlapping wild-type and mutant traces.

The Heterozygous INDEL Deconvolution tool separates the mutant trace from the wild-type trace to detect the INDEL variation and quantification as shown in Figure 6. The entire tool is interactive, variations can be edited, and images can be printed at high resolution.

Figure 6: Heterozygous Deconvolution Tool
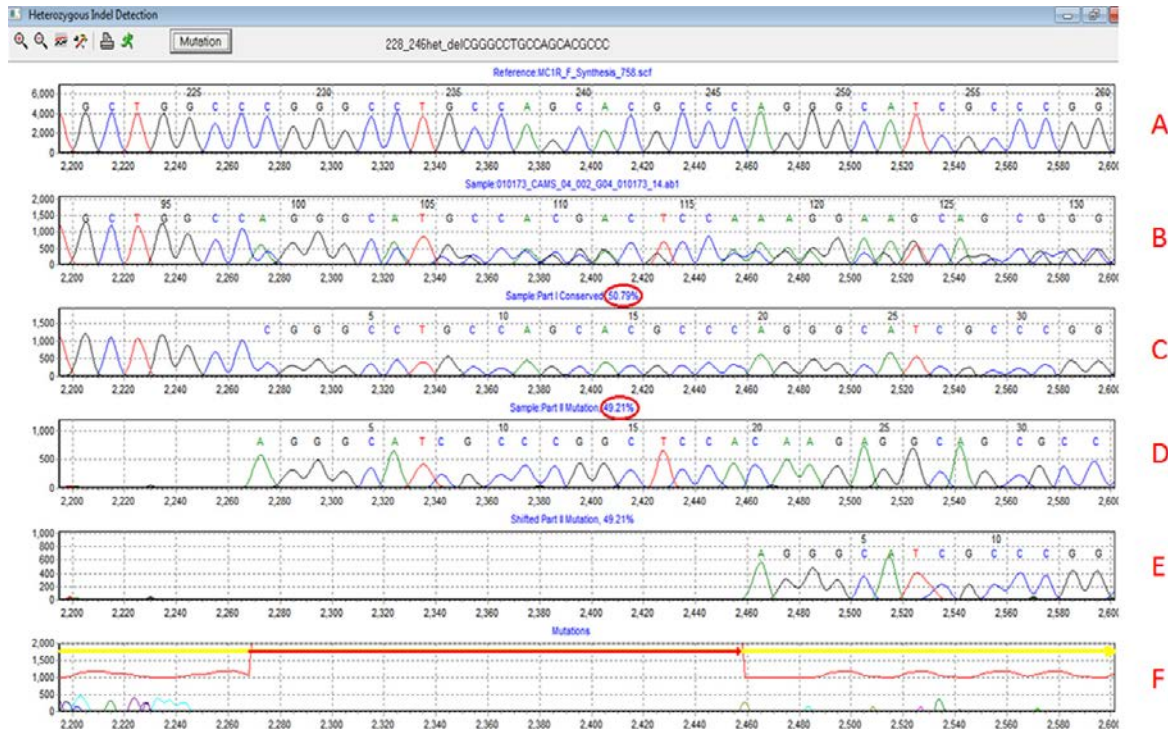
This illustration shows the detection of a 19bp heterozygous deletion. (A) is the reference trace, (B) is the sample trace, (C) is the wild-type trace aligning to the reference, (D) is the deconvoluted mutant trace, (E) is the shifted mutant trace aligning to the reference, and (F) is the mutation electropherogram that indicates the position and type of INDEL. The mutant percentage is estimated by taking the ratio of the mutant intensity over the total intensity and reported, as indicated by the red circles in Figure 6.

Low Frequency Variant Detection and Quantification
(Somatic Mutation, Heteroplasmy, and Mosaicism)

Low Frequency variations are often difficult to detect as they can be buried in the baseline of the sequence trace. These mutations include somatic mutations, heteroplasmy (usually in mitochondria) and mosaicism. Mutation Surveyor software contains a special algorithm to detect and display these possible low frequency variations.

Figure 7: Low Frequency Variant Detection
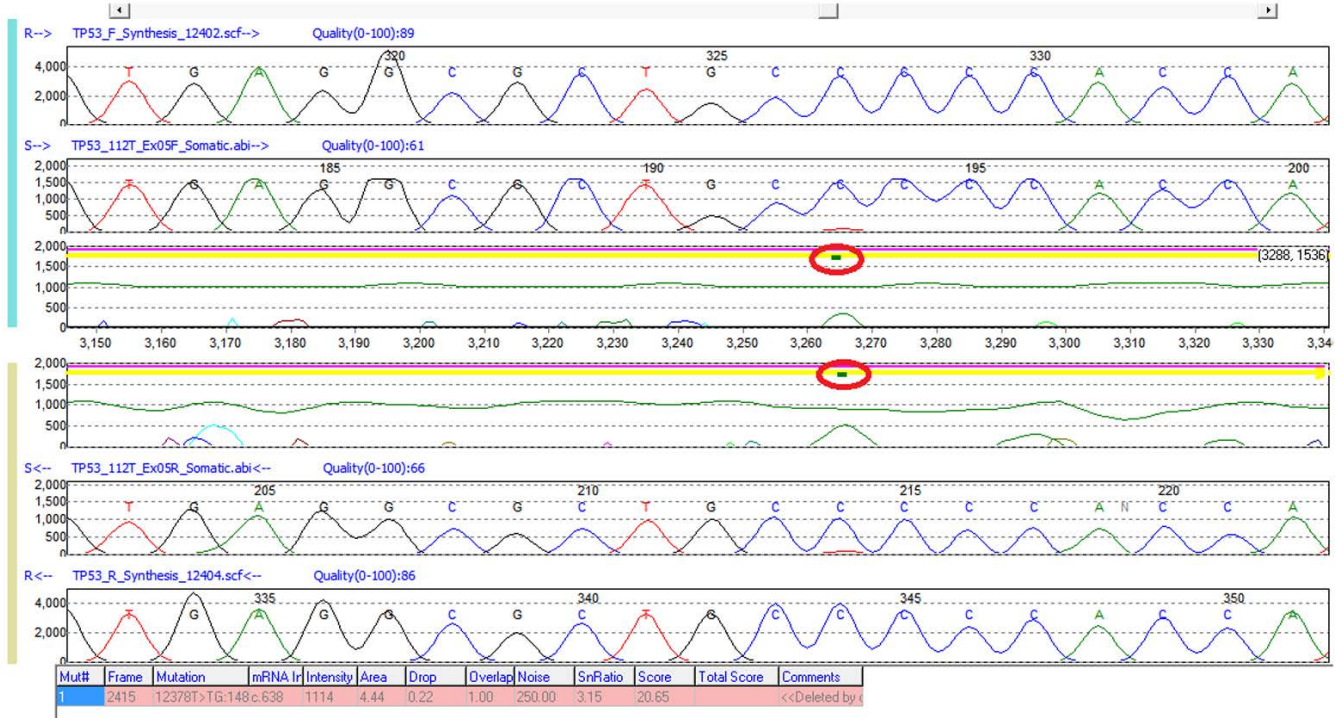
A possible somatic mutation detected in the tp53 gene. The green bar in the mutation electropherograms indicates the position of the possible somatic variation. The software detects these low frequency variations by looking at both the forward and reverse traces. If it detects a minor component of the same wavelength at the same position in both traces, it will alert the user to that position with the green bar.

Mitochondrial DNA Sequence Analysis

The mitochondrial genetic code has a few key differences from the human genetic code. Mutation Surveyor software will automatically switch to the mitochondrial genetic code if a mitochondrial GBK file is loaded. It will use this genetic code to show the correct amino acid change when analyzing the sample sequences.

Mutation Surveyor software can automatically read a tag in the GenBank file to recognize the mitochondrial genetic code and use it for the analysis without the need for any additional steps. The software can also display multiple CDS, as shown in Figure 8 (indicated by the yellow line), for the amplicon if annotation is available in the GBK file. The start and end position of the mitochondrial's circular genome is indicated by the double sided arrow in Figure 9. The amplicon in the illustration spans the origin of the genome and the sequence can match continuously.

Figure 8: Multiple CDS of the Mitochondrial Genome
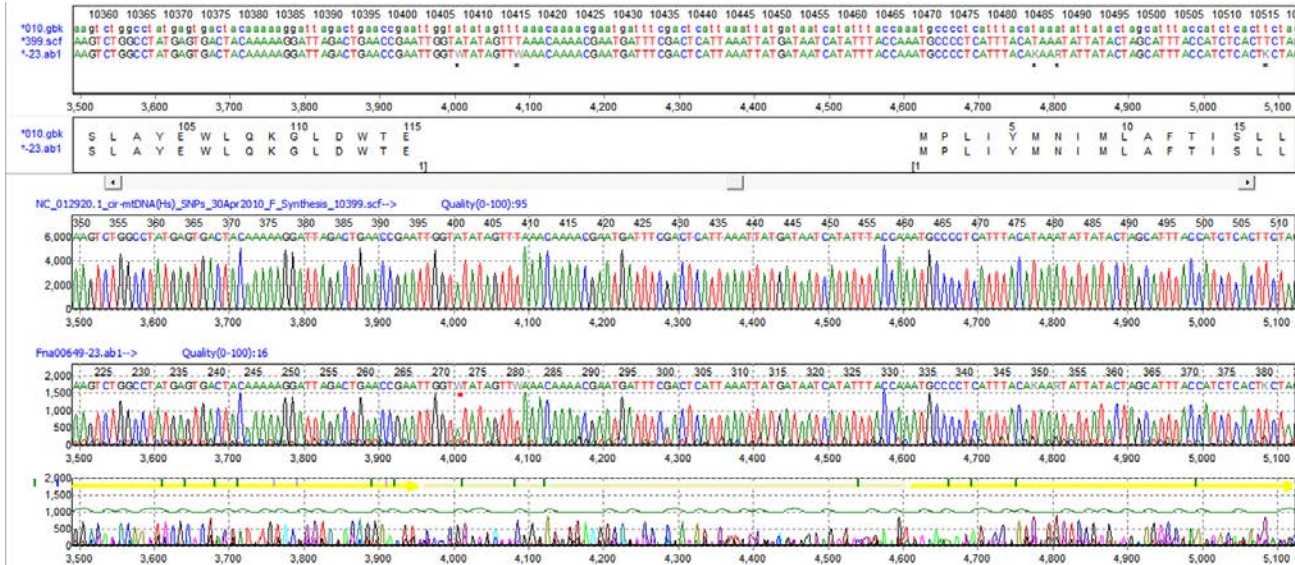
Mitochondrial sequence trace spanning multiple coding regions. Each CDS is displayed with a yellow bar.

Figure 9: Origin of Mitochondrial Genome
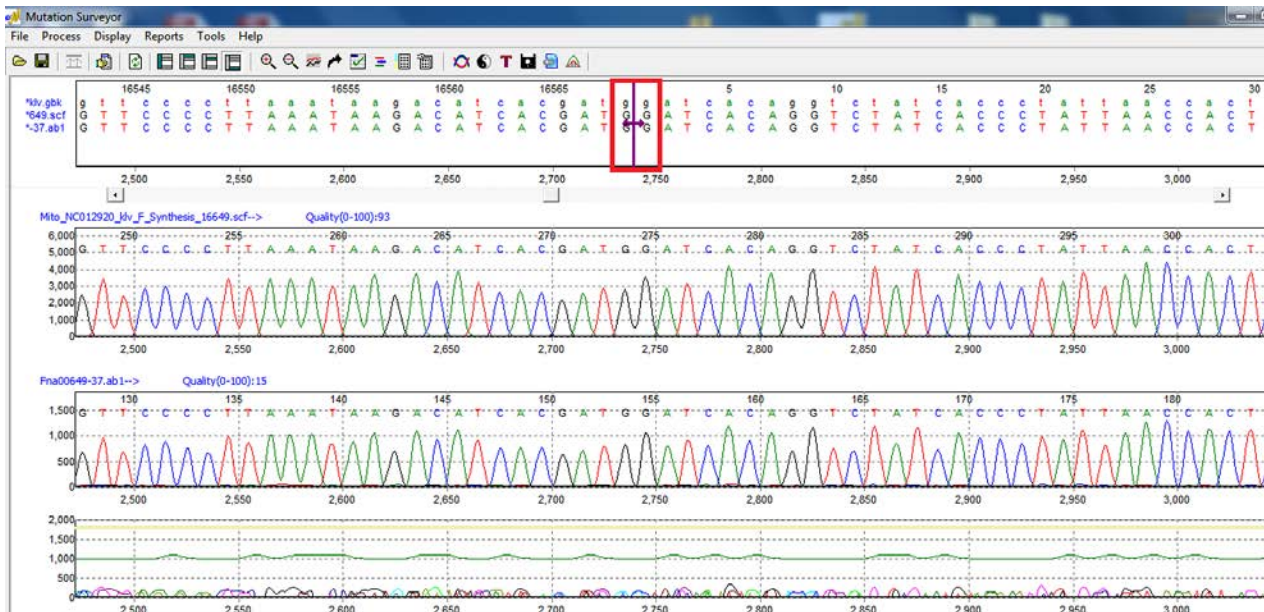
Mitochondrial sequence trace spanning the overlap region. The start and end position of the mitochondrion's circular genome is indicated by the double sided arrow.

Methylation Analysis

Mutation Surveyor software has the option to analyze sequences that have gone through bisulfite treatment for methylation studies. After sequencing, the resulting files can be imported straight into Mutation Surveyor software for analysis. The software will automatically identify methylated sites, successful, unsuccessful and partial conversion of Cytosine to Uracil sites during bisulfite treatment. It takes one click to activate the methylation analysis parameters.

Figure 10: Methylation Analysis Display
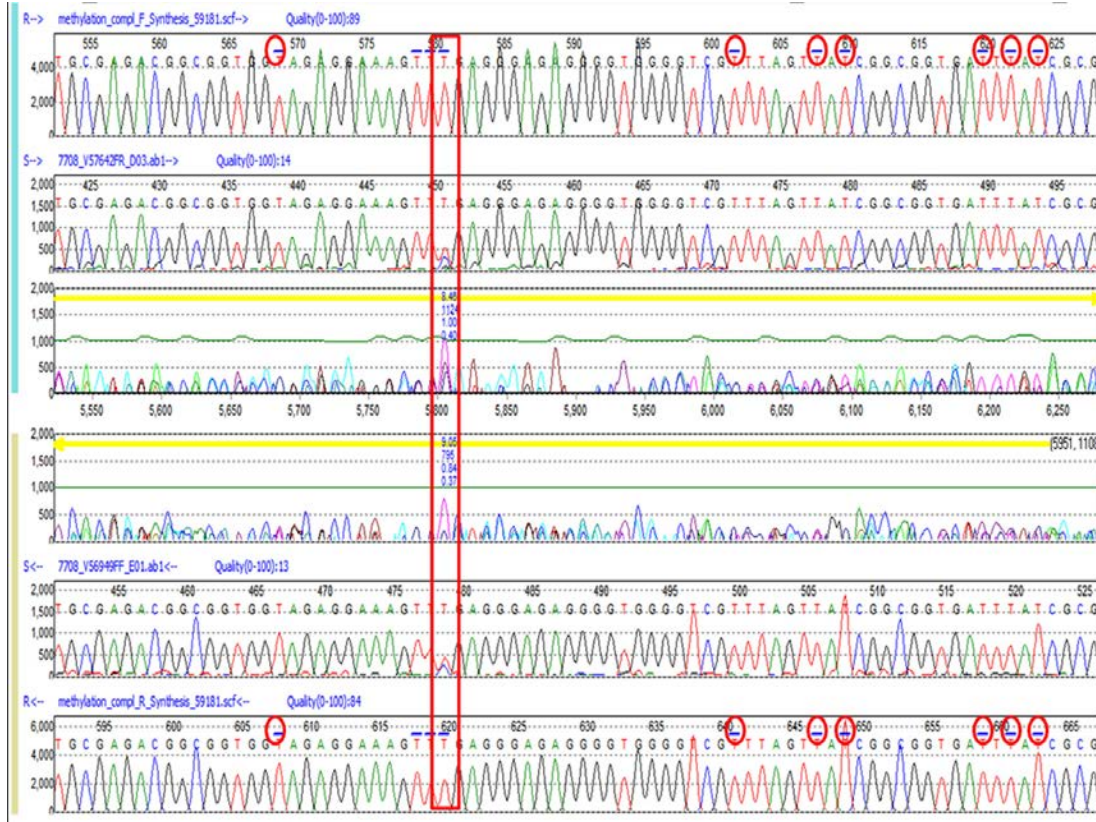
Methylation analysis in Mutation Surveyor software displays the positions that used to be a cytosine with a blue bar on top of the position. The indicated variation shows incomplete conversion of cytosine to uracil at that position.

Mutation Surveyor software has a specialized methylation report that indicates all positions that are methylated, successfully converted, partially converted, and unsuccessfully converted. In Figure 11, the samples are listed at the top row, positions and nucleotides are in the first column. You can double click on any cell in the report and it will take you to the specific position in the main analysis window for quick review. This report can be printed, saved, or exported as a text or Excel format file.

Figure 11: Methylation Report

			7373_V56949FF_B01.ab1	7373_V57642FR_A03.ab1	7708_V56949FF_E01.ab1	5505_V56949FF_C01.ab1	7708_V57642FR_D03.ab1
68876	G	g	-	-		-	
68877	G	g	-	-		-	-
68878	G	g	-	-	-	-	-
68879	G	g	-	-	-	-	-
68880	T	t	-	-		-	-
68881	C	t	S	S	S	S	S
68882	C	c	M	M	M	M	M
68883	G	g	-	-	-	-	-
68884	C	t	S	S	S	S	S
68885	C	t	S	S	S	S	S
68886	T	t	-	-	-	-	-

The methylation report indicates positions that are methylated, successfully converted, partially converted, and unsuccessfully converted.

Hypervariable Sequence Analysis

Using Mutation Surveyor software's multi-step alignment algorithm, the software aligns hypervariable sequences to the correct reference for mutation detection. The Graphic Analysis Display shows all called mutations, a mutation table, amino acid changes and positions of differences for easy reviewing and interpretation. This application allows users to analyze virus DNA sequences, HLA typing, and many other hypervariable sequences.

Figure 12 shows a HCV sample with high mutation frequency aligning to the reference for mutation detection. Variations in the sample that causes a change in the amino acid can be seen in (1) the amino acid pane. The mutation electropherogram (2) indicates positions of differences between the two traces with a peak at the location. Each mutation call is recorded in the mutation table (3) at the bottom. For further clarification, the GenBank pane (4) also displays the nucleotide differences with an asterisk at the bottom of the pane.

Figure 12: Hypervariable Sequence Analysis
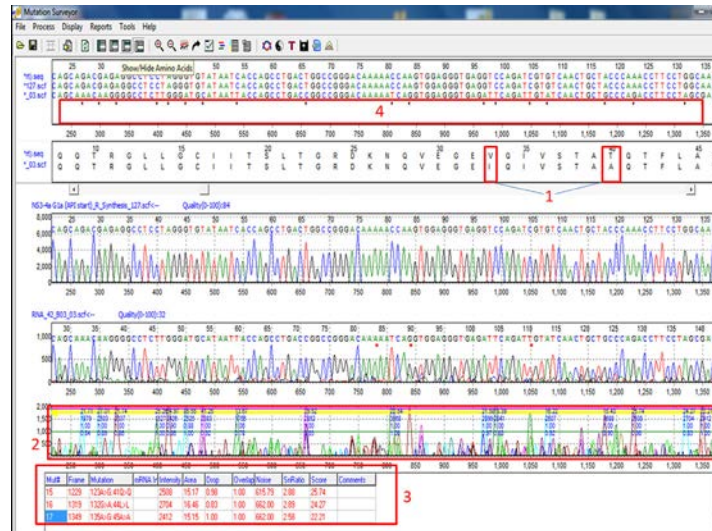
HCV sample with high mutation frequency aligning to the reference for mutation detection.

Mutation Quantification

Mutation Surveyor software has a built-in Mutation Quantification tool to eliminate the need to transfer data from one software package to another. This tool offers two methods of quantification, Standardized Allele Ratio and Simplified Allele Ratio.

Standardized Allele Ratio will use two standards to quantify variations of interest: mutant and wild-type standards. In Figure 6, the drop intensity of the "C" nucleotide is about 30.61% while the gain in the "G" intensity is about 31.66%. Standard 1, the top pane, is the wild-type sequence. Standard 2, the bottom pane, contains the 100% homozygous mutant sequence. The middle pane contains the test sample.

Figure 13: Standardized Allele Ratio
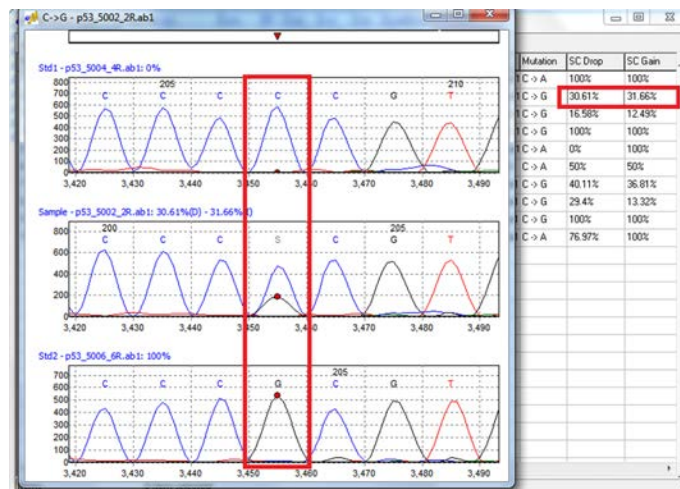
User Management and Audit Trail

Mutation Surveyor software includes a user management system to control access rights and to generate a user audit trail for each analysis project. Access rights for each user are determined by the administrator, providing assurance that unauthorized individuals do not accidentally alter a project or change the analysis parameters established by the laboratory. User management also provides user ID and Organization in the header of the final reports. Each change to the project is captured by user and time stamped.

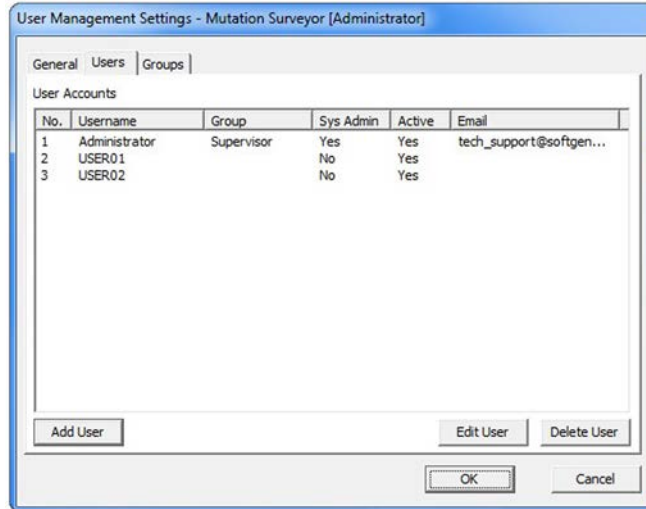**Figure 14: User management settings**
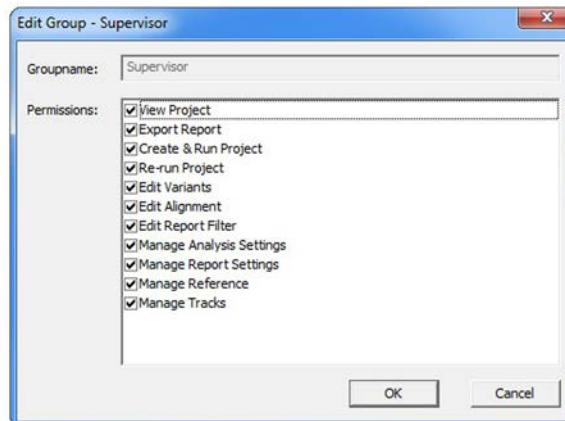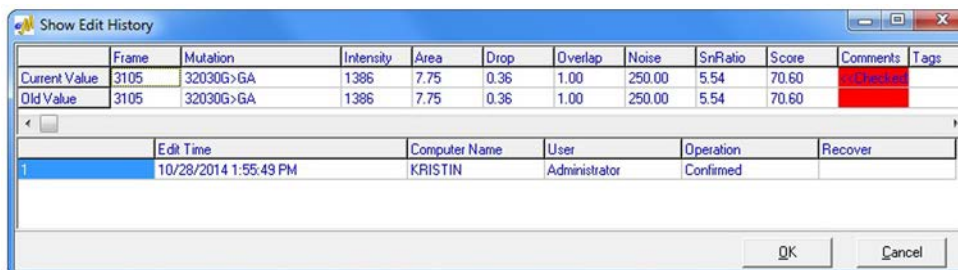

Figure 15: User permissions

**Figure 16: Edit history of mutation call showing time of edit and user name**

GenBank and SEQ File Editors

Mutation Surveyor software package also comes with GenBank and SEQ file editors so that Regions of Interest and variants can be added for analysis. These files can also be extracted from projects to be used for future references.

The Advanced GBK File editor can open any GenBank file downloaded from NCBI. In this dialog, the user can choose specific isoforms that will be used for the analysis, create Regions of Interest, and add variants to the variation list.

Figure 17: Advanced GBK File Editor
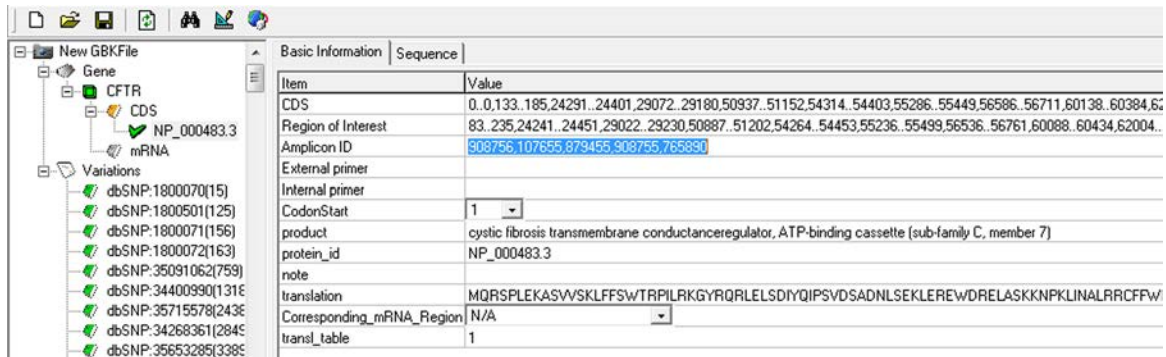
Advanced GenBank File Editor can add specific Regions of Interest or have the software automatically create ROIs based on CDS. Amplicon IDs can be added in the Amplicon ID field. The variations in the GenBank file can also be edited by adding, deleting, or editing an existing variation as needed.

Figure 18: SEQ File Editor
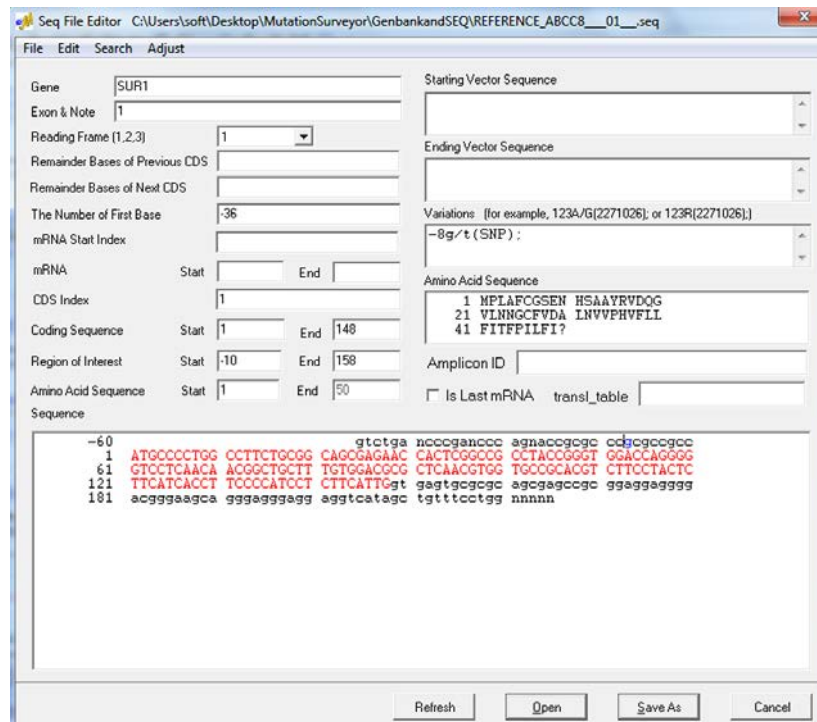
The SEQ File Editor allows the user to import individual SEQ files for editing. In this dialog box, the user can specify the ROI, CDS, Amino Acid, Variations, etc... This tool is used to create individual SEQ files for a single amplicon to be used for mutation detection. After saving, the file can be imported directly into the software.
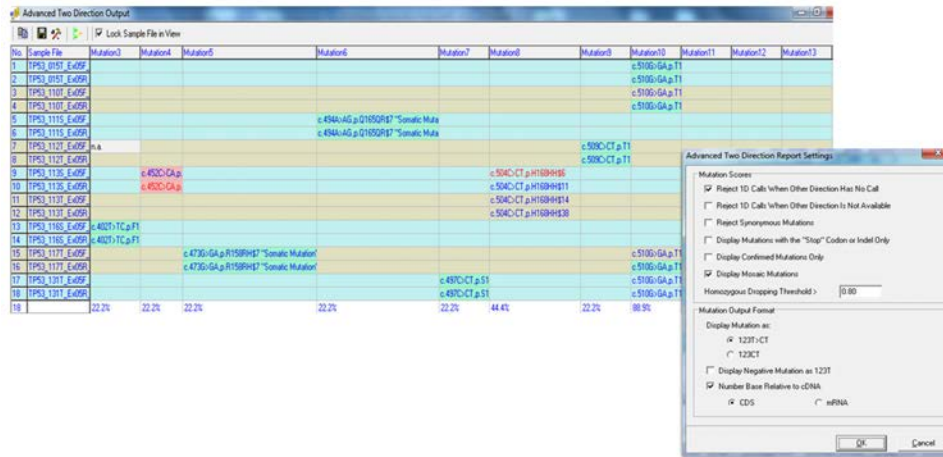
Reporting Options

Mutation Surveyor software offers a variety of reporting options including an entirely customizable report. The types of reports range from unique analysis, such as the Methylation Report, to more generalized reports for 2-directional data. Some of the reports contain color-coding for variant tags, grouping options, and filtering options for different types of variations. Reports are linked to graphics in order to easily view variants of interest.

Advanced 2-Directional Report

The Advanced 2-Directional Report has options to ignore calls only made in one direction as they tend to have a lower confidence. Within the report, there are different display and filtering options.

Figure 19: Advanced 2-Directional Report

Custom Report

The Custom Report is the most versatile report in Mutation Surveyor. It has options to customize everything from grouping of the samples to which type of mutations are displayed, as shown in Figure 20. It also has mutation call filtering options, such as filtering by coding sequences (CDS) and regions of interest (ROI). In addition, there are color-coding options, shown in Figure 21 and 22, for variant tags associated with variations detected for easy identification. The report can be printed or exported as a text, Excel, XML, or VCF format for input into Geneticist Assistant NGS Workbench.

Figure 20: Mutation Filter Options

Figure 21: Mutation Color Code Options
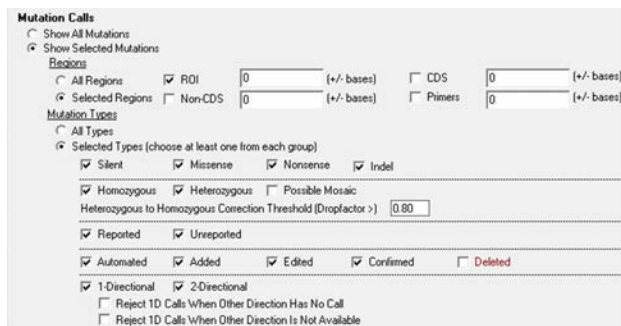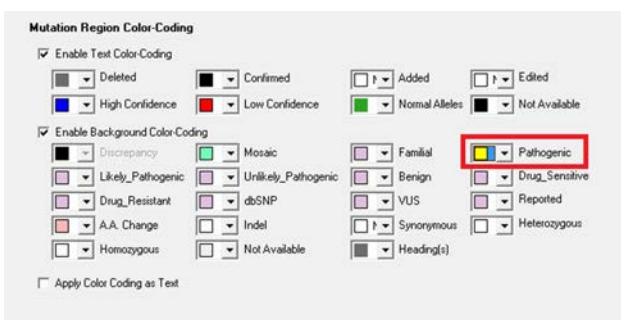

Figure 22: Custom Report Display

Variant detected in the illustration has a "pathogenic" tag. This specific tag was selected to be color-coded yellow in Figure 22 and shows up in the report with a yellow background.

VCF Report

The Custom Report of Mutation Surveyor software also offers the option to export genetic sequence variations in Variant Call Format (VCF). This flexible format can be adapted to store a wide variety of information and can be used in conjunction with other software tools including Geneticist Assistant™ NGS Interpretative Workbench. Using VCF output, Mutation Surveyor software users can quickly and easily input results into Geneticist Assistant for confirmation, variant interpretation and functional prediction.

Clinical Report

The clinical report has been formatted to only display samples that contain mutation calls or reported variants based on the GenBank file. The report allows for a custom header that may include the institution name, address, and other information.

Figure 23: Clinical Report

Clinical Report is displaying the header information at the top and the mutation calls made in the sample as well as the sample names. Each page of the report refers to a different sample and the mutation calls made in those samples. This report can be printed as high-resolution images.

Other Reports Include:

- Mutation Report
- Project Report
- HGVS Report
- Graphic Display Report
- GAD Report
- Full Gene Report
- PrettyBase Report

Reference Assembly

The Project Reviewer Tool can perform reference assembly on replicate samples or overlapping amplicons that align to the same reference. Users can view the entire project with multiple electropherograms and samples in the same window.

Figure 24: Overlapping Amplicons in Project Reviewer Report
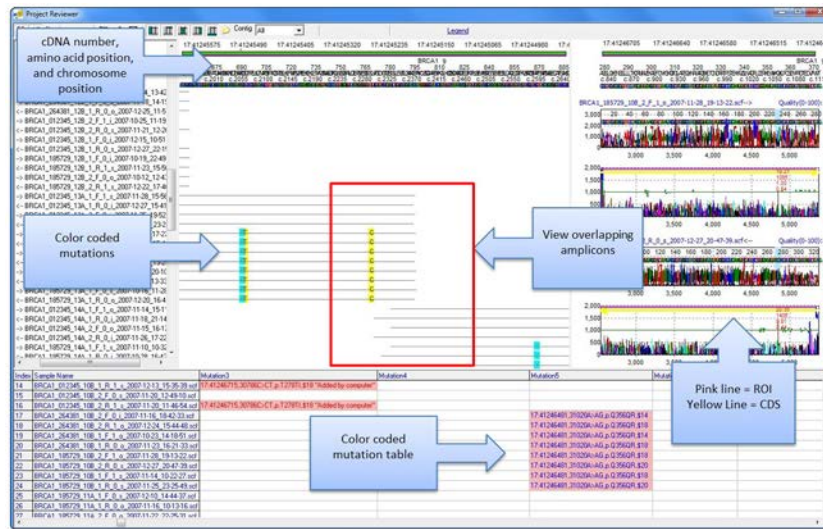
Project Reviewer: main analysis window showing overlapping amplicons of a single patient with color coding of variants. c.DNA and chromosomal positions are shown with the nucleotide string. Users can customize the display by choosing which panes to use by using the toolbar at the top of the Project Reviewer.

The Project Reviewer also has a grouping tool that allows the grouping of sequences by characters or by amplicon ID. In Figure 25, the illustration shows replicate samples grouped by patient IDs.

Figure 25: Replicate Samples in Project Reviewer Report
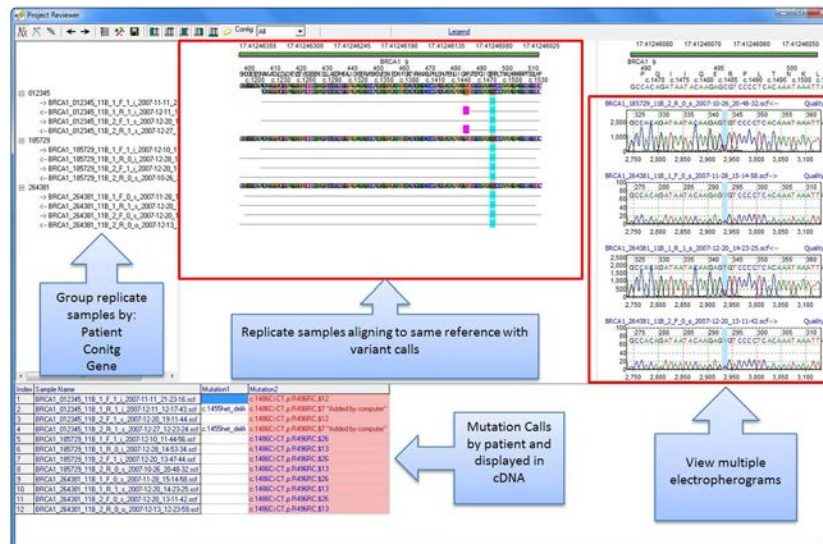
Project Reviewer showing replicate samples for three different individuals aligning to the same reference. The samples are grouped by patient ID. Variants are detected at the same positions in all three patients. Multiple electropherograms can be seen at one time to compare mutation calls between samples. The mutation table at the bottom lists all mutations for the selected patient.

Recommend Hardware Requirements

Please open disk to review your applications of interest and to install a 30-day trial of Mutation Surveyor software.

Disk Contains:
Application Notes
30-day free Mutation Surveyor Trial
User Manual

Mutation Surveyor software has been tested and validated for various computer systems. The minimum system requirements are:

Windows® PC
OS: Windows Vista, Windows 7, Windows 8, Windows Server 2008 R2, Windows Server 2012 R2
Processor: dual-core, 1.8GHz
RAM: 2 GB
Available hard disk space: 60GB

Intel® Powered Macintosh®
Parallels® desktop for Mac (Mac OS/virtual machine dependent) or Apple™ Boot Camp or VMware® Fusion
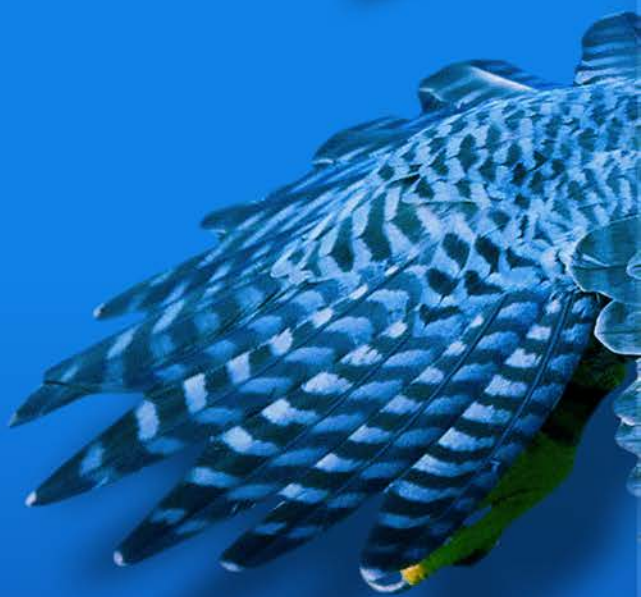(Mac OS/virtual machine dependent)
RAM: 2GB
Available hard disk space: 60GB

Installation of Mutation Surveyor software is not supported on Linux or UNIX-based operating systems.

If trial disc is not present please email info@softgnetics.com
for a free 30-day trial

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